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Effect of solid-state fermentation on Anthocyanin and physicochemical content of Lebui bean (*Cajanus* sp.)

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Solid-state fermentation (SSF) is a fermentation method using a solid or dry substrate and less water. The SSF process is very important to get food products that contain high bioactive compounds and increase the nutritional value. Anthocyanin is the important bioactive compounds and one of the largest components of phenolic group contained in the Leguminosae. Lebui bean (*Cajanus* sp.) is one of the most important black seed legume at Lombok Island West Nusa Tenggara Indonesia. Lebui bean is very potential as a source of nutritional compound and bioactive compound especially anthocyanin. Anthocyanin in lebui bean is present in the cell which are bound with glucose through glycosidic bond and therefore should be degraded by the used of microbes through fermentation such as by SSF method. Functional properties of fermented lebui bean produced by *Lactobacillus* sp. and *Rhizopus* sp. by SSF method were also determined. The aim of this study was to investigate the best type of microbe and fermentation time through SSF method to produce lebui bean powder with the highest levels of anthocyanin and nutrient components. A nested design with two factor was used. The main factor is the type of microbe (*Lactobacillus* sp. and *Rhizopus* sp.) and the second factor is the fermentation time (1, 2, 3, and 4 day) nested on the main factor. The result showed that fermentation carried out for two days with *Rhizopus* sp. as a microbial culture is the best treatment in this study. This treatment produce lebui bean powder containing anthocyanin, total protein, fat, crude fiber, carbohydrate, water and ash were 154.70 ppm, 22.73%, 0.54%, 8.07%, 66.78%, 6.76%, and 3.19%, respectively.

Keywords: solid state fermentation, anthocyanin, *Lactobacillus* sp., *Rhizopus* sp.

INTRODUCTION

Solid-state fermentation (SSF) is a fermentation method using a solid or dry substrate and less water. The SSF fermentation method has several advantages such as simple process, energy saving (Prado et al. 2005), high productivity, high product stability (Hölker and Lenz, 2005), and suitable to apply for *Leguminosae* or cereals to improve quality nutrition (Chawla et al. 2017), total anthocyanin and total phenolic compounds (Lee et al. 2008).

Lebui bean (*Cajanus* sp.) is one of the most important legume grown throughout the year and had been found abundantly at Lombok Island West Nusa Tenggara Indonesia (Lombok Agricultural Service, 2011). Lebui beans are only used as local dishes and traditional medicine by Lombok people. In peak season, lebui beans are unutilized and only left in the field. Whereas, lebui bean is very beneficial as local material to produce high valuable bioactive compounds such as anthocyanin. The anthocyanin compounds are

part of the phenolic components found in black beans of *Phaseolus vulgaris* L. (Xu and Chang, 2009) and koji fermentation (Lee et al. 2007). This potential bioactive compound has an advantages to cure some infectious diseases such as skin infection, gastroenteritis, pneumonia, due to this lebui bean has secondary metabolites product and serves as antioxidant, antimicrobial, and antifungal (Akor and Anjorin, 2009; Zafar et al. 2014). However, these bioactive compounds present in the cell which are bound with glucose through glycosidic bond (Huang et al. 2013) and this linkage should be degraded by the used of microbes through fermentation and the effective method is SSF method.

MATERIALS AND METHODS

Raw materials

Lebui beans (*Cajanus* sp.) were collected from Lombok Island, West Nusa Tenggara Indonesia. These beans were washed thoroughly with water to remove the dirt's, then immersed into water (ratio of 1:3), allowed to stand for 6 h in 25-27°C, and the water was replaced for every 1 h. The beans were filtered and coarsely grounded up to 1-2 mm in size, then dried by using cabinet dryer at 40°C for 6 h until the moisture content reached 12-13%, then finely grounded to 50 sieve mesh size and we sieved it to obtain the lebui bean powder (LBP).

SSF procedures

The LBP is fermented using a microbial culture of *Lactobacillus* sp. (L) or *Rhizopus* sp. (R) adjusted for each treatment for 1 to 4 days. The LBP is heated in cabinet dryer at 70-80°C for 15 minutes. About 2% dry culture of L or R were added to LBP, then fermentation is carried out for 1 to 4 days according to the treatment at 27-28°C. Drying is done on the cabinet dryer at 40°C for 5 h, then ground into a fermented LBP and stored in a tightly closed glass container.

General experimental procedures

A nested design with two factor was used. The main factor is the type of microbe (*Lactobacillus* sp. and *Rhizopus* sp.) and the second factor is the fermentation time (1, 2, 3, and 4 day) nested on the main factor.

Observation parameters were total anthocyanin, protein, fat, moisture, ash, carbohydrate, and total fiber. The proximate contents analysis were measured by AOAC (2000) i.e. water content, protein content, ash content, and fat content. Total anthocyanin was determined by Juniarka et al. (2011). The research data were analyzed using nested ANOVA. The present work studied the influence of solid state fermentation by *Rhizopus* sp. and *Lactobabillus* sp. on the functional properties of lebui bean intending to provide information about their use in food products formulation

RESULTS AND DISCUSSION

Total anthocyanin

The purpose of the LBP fermentation process is to decompose the glycoside bonds that bind the bioactive compounds, so that the bioactive compounds will become free forms including anthocyanins. Bioactive compounds in fermented products have a low level of damage because the fermentation does not use high temperatures that can damage these bioactive compounds. Fermentation also aims to improve the nutritional quality of beans.

The anthocyanin content of fermented LBP with R was in the range 122.92-154.70 ppm, whereas fermented with L had lower level was 14.77-137.71 ppm. The anthocyanin levels in LBP fermented with R or L tend to decrease with increasing length of fermentation time. This condition is in line with Balik (2006), during fermentation can occur biochemical, metabolism, degradation, and destruction process conducted by microbes to produce more sugar components to support its growth, resulting in decrease of non-sugar components and secondary metabolite compounds including anthocyanin. Longer fermentation will result in increased microbial mass and positively correlated with decreased levels of anthocyanin in fermented products.

The decrease in anthocyanin levels during the fermentation period was also reported by Afoakwa et al. (2012). During fermentation, anthocyanin levels may decrease with increasing length of fermentation time and shown by decreasing the level of dark color of the extract into lighter colors. Anthocyanins are polyphenol components and highly sensitive to changes in temperature and pH. Therefore, the longer time of fermentation and the presence of environmental influences will result in a decrease of anthocyanin levels (Hornedo-Ortega et al. 2017).

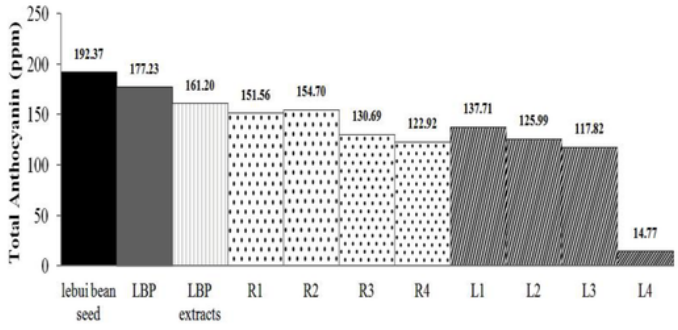

Figure 1. Total anthocyanin of LBP. R: *Rhizopus* sp. L: *Lactobacillus* sp. Fermentation time (1, 2, 3, 4 day)

The decrease in anthocyanin levels during the fermentation period was also reported by Afoakwa et al. (2012). During fermentation, anthocyanin levels may decrease with increasing length of fermentation time and shown by decreasing the level of dark color of the extract into lighter colors. Anthocyanins are polyphenol components and highly sensitive to changes in temperature and pH. Therefore, the longer time of fermentation and the presence of environmental influences will result in a decrease of anthocyanin levels (Hornedo-Ortega et al. 2017). Hydrolysis of the anthocyanin to anthocyanidin and then into a simple sugar group during the fermentation of plant seeds will result in a decrease of anthocyanin levels. The decline of these anthocyanin levels will continue to occur and peak on the 36 day of fermentation (Afoakwa et al. 2012). Based on the results of experiment, fermentation treatment up to 2 days gave better results than day 3 and 4. This relates to the occurrence of changes after more than 2 days of fermentation caused by the increase in temperature in the material. This increase in temperature will result in odor changes, discoloration, and anthocyanin levels, either fermented with R or L.

Physicochemical properties

Protein content

There is an influence between microbial type and duration of fermentation nested on microbial type to LBP protein level is shown in Figure 2. The average of protein content of LBP fermented with R or L is higher than non-fermented LBP is 18.65-22.73% higher than 18.53%. This proves that LBP fermented with L or R, can produce a final product that has higher protein content. Igbabul et al. (2014) stated that the process of fermentation of the mahogany bean (*Afzelia africana*) up to 72 h can increase the protein content from 21.88% to 22.43-26.8%. The fermentation process can lead to hydrolyze of protein molecules, resulting in amino acids and nitrogen.

Increased levels of this protein according to Kasprowicz-Potocka et al. (2016) is due to an increase in the mass of microbes that will hydrolyze the protein substrate to produce free amino acids and nitrogen to support its growth. Handoyo and Morita (2007), Engel et al. (2011) and Meussen et al. (2012) stated that the optimal pH for the growth of *Rhizopus* sp. is about 3,6 to neutral pH. Therefore, mould growth and hydrolysis process of protein component will decrease along with increasing pH and fermentation time.

Fat content

The average fat content of LBP fermented with R and L, respectively are 1.13% and 1.44%. Fermentation using culture R showed a difference in fat content between fermentation time 1, 2, 3, and 4 days. However, fermentation with L showed no significant difference between fermentation time of 1, 3, and 4 days, but the results of the three treatments were significantly different from those fermented for 2 days. The highest fat content in fermentation with R and L respectively was 0.756% achieved within 4 days and 1.545% achieved within 3 days, while the lowest was 0.54% and 1.32% after fermentation for 2 day.

There was no significant difference in LBP fat content fermented with L until 4 days of fermentation and occurred along with the changes of protein levels.Osman (2011) also stated that during fermentation there was no noticeable change in the fat content of the final product and this was also related to the low fat content of various plant seeds.

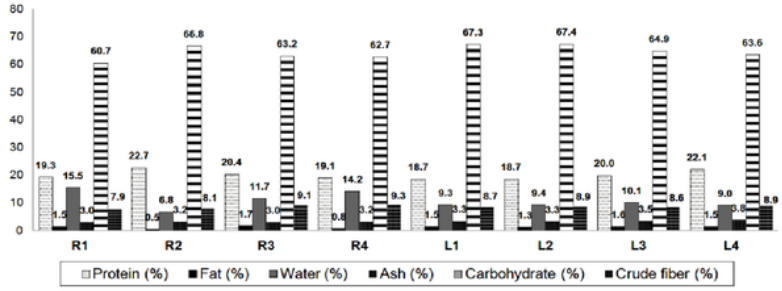

Figure 2. The nutritional value of fermented LBP. R: *Rhizopus* sp. L: *Lactobacillus* sp. Fermentation time: 1, 2, 3, 4 day

The decrease in fat content on the second day of fermentation, is possible because of the initiation stage of microbial growth, therefore the fat molecules that have been hydrolyzed into simpler compounds are used to support the microbial life.

On the 3rd day, there was an increase in the fat content caused by the number of simple fatty acid compounds produced by microbial hydrolysis and the breakdown of the lipoprotein complex of the material, but the fatty acid has not been used entirely by microbes to support its metabolism (Oliveira et al. 2011; Niveditha et al. 2012). On day 4, the fat content also decreases.

This decrease occurs in LBP fermented with R or L. The decrease in fat content is also reported by Khetarpaul and Chauhan (1989) which occur during fermentation up to 72 h. The hydrolysis of fat molecules due to the increased activity of lipolytic enzymes produced by microbes during fermentation has resulted in decreased levels of product fat until the end of fermentation time (Obadina et al. 2013).

Water content

During fermentation with R or L, the water content of the product decreases or increases followed by an increase or decrease in the value of other nutrients. The average water content of LBP fermented with R is 6.76% -15.49%, whereas fermented with L shows a higher value is 9.02% - 10.12%. Orhevba (2011) and Obadina et al. (2013), there is a decrease in water content in the first 54 h of fermentation and then the water content increases until the end of fermentation. Along with the decrease in water content, protein and carbohydrate levels of LBP fermented with R increased, respectively 19.31% to 22.73% and 60.66% to 66.78%. The same condition was also reported by Morris et al. (2004). The high water content of fermentation with R is also due to the relatively high of CO_2 production that generated during the fermentation process and reentering to the product as water points especially in the surface of product, thereby increasing the water content of the final product. Control of excess water content at the time of LBP fermentation is by aeration holes and performing a top-down reversing every 12 h. Nout and Kiers (2005) suggested to provide sufficient distance between the products and regulate the thickness of the fermented product, thereby the heat generated by the mass of *Rhizopus* sp. will not reach a maximum temperature of 40-50°C and the ambient temperature is still suitable for its growth.

SSF method used in this research has been able to avoid the fermented LBP become putrid because of high water content. Water is only used to mix LBP with microbial cultures. Therefore, LBP that has been fermented for up to four days does not indicate putrefaction and does not present a strong odor.

Ash content

Average ash content (%) LBP fermented with R and L respectively R3 <R1 <R2 <R4 is 3.01 <3.02 <3.19 <3.20 and L2 <L1 <L3 <L4 Is 3.27 <3.33 <3.49 <3.82. The ash content of LBP fermented with L (3.48%) was higher than that fermented with R (3.10%). The overall ash content of the fermented LBP is lower than 3.5% and meets the standards for food preparations made from the seeds of a plant (Pomeranz and Clifto, 1981). The ash content of fermented LBP has a value that is not different from the whole lebui bean and unfermented LBP of 3.37% and 3.45%, and is not different from the ash content of other beans in the *Leguminosae* group that ranges from 2.02-9,36% (Megat Rusydi, et al. 2011) and 3.5-3.9% (Wani et al. 2017). This shows that the ash content contained in lebui bean ranges from 3 to

3.5%. Osman (2011) reported that fermentation does not have a sharp effect on the decrease or increase in ash content of the final product. According to Difo et al. (2015), a decrease in ash content is caused by a decrease in some mineral content in materials such as Fe, Na, Mg, Zn, and K used by microbes to support its growth during fermentation.

Carbohydrate content

Carbohydrate levels of LBP fermented with L have a higher value than those fermented with R. The average carbohydrate content of LBP is 60.66-67.4%. During fermentation for the first 24 h, there has been a decrease in carbohydrate levels for all treatments from 68.91% to 60.66% for LBP fermented with R and to 67.32% for LBP fermented with L. The decrease in carbohydrate levels appears after the 2nd day of fermentation until the 4th day of fermentation for both types of microbes. The declining trend in carbohydrate levels is also indicated by the Osman (2011). Decreased carbohydrate levels are caused by microbial enzymatic activity and begin to appear in the first 24 h of fermentation. At 12 to 24 h of fermentation, a low pH condition can inhibit the action of amylase released by microbes and this condition also results in the release of antimicrobial compounds such as terpenoid groups, thus the number of microbes is still controlled and carbohydrate levels have not shown a decrease.

Crude fiber content

The fermentation process can increase the crude fibre content from the initial content in the LBP 7.89% to 8.59% with R treatment and 8.78% with L treatment. Fermented LBP crude fiber content tends to increase with length of fermentation time until the end of day 4, whether fermented with R or L. The high levels of crude fiber to the end of fermentation indicate that cellulase enzymes produced by both types of microbes have been able to degrade cell walls in bean powder into simpler components including crude fiber components. This condition is in line with research conducted by Mirnawati et al. (2012) which reveals the function of cellulase enzymes in degrading the cell wall of materials with hard outer skin such as palm kernel, thereby increasing the crude fiber content. Based on the results of Mirnawati et al. (2013) by *Eupenicillium javanicum* fungus in fermentation, a decrease in cellulose and crude fiber in the fermentation process may indicate that the longer fermentation time will lead to an increase in the amount of microbial mass, but the cellulase enzyme produced by these microbes is used to degrade cellulose to into glucose and used as an energy source to support its growth.

CONCLUSION

The SSF method used in this study has been able to increase the nutrient content of LBP, however the anthocyanin levels have decreased. Decreased levels of anthocyanin are quite low, therefore the SSF method is suitable for use in increasing nutritional content and maintaining anthocyanin levels in lebui bean (*Cajanus* sp.). Fermentation carried out for two days with Rhizopus sp. as a microbial culture is the best treatment in this study.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

WM, SK, W, and IS has performed the experiments and also write the manuscript. WM prepared the extract. All authors read and approved the final version.

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